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PGC-1a Therapy for Parkinson Neurodegeneration

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14. ABSTRACT
Currently there are no natural disease-modifying treatments for Parkinson's disease (PD). Microarray analyses have implicated decreased expression of proliferator-activated receptor gamma co-activator-1 alpha (PGC-1α) as a potential pathobiologic link to the mitochondrial dysfunction in Parkinson's disease (PD). *In vitro* and *in vivo* studies show that PGC-1α deficiency increases the susceptibility to MPTP-induced neurotoxicity, and enhanced PGC-1α levels protect neural cells from oxidative stress or α-synuclein-mediated neuronal death. Collective evidence to date, therefore, strongly suggests that PGC-1α is a promising therapeutic target within the central nervous system (CNS) for the treatment of PD. Herein we hypothesize that pharmacologically titrated upregulation of PGC-1α will provide protection against neurodegeneration in PD. Our goal is to modify disease progression by modulating PGC-1α levels with CNS-targeted small molecule therapeutics at an early stage of PD pathogenesis. We have identified that fenofibrate, an FDA approved drug, induces PGC-1α expression in CNS cells and exerts both cytoprotective and anti-inflammatory effects. Given the demonstrated safety profile of fenofibrate *in vivo*, we will test whether fenofibrate offers therapeutic potential in PD models as a prelude to a potential clinical study in patients. In this reporting period, we have demonstrated that fenofibrate mediates anti-inflammation in a PGC-1α-dependent, PPARα-independent manner. We further evaluated in vivo efficacy of fenofibrate in a MPTP mouse model of PD.

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Introduction

Currently there are no natural disease-modifying treatments for Parkinson's disease (PD). Microarray analyses have implicated decreased expression of proliferator-activated receptor gamma co-activator-1 alpha (PGC-1α) as a potential pathobiologic link to the mitochondrial dysfunction in Parkinson's disease (PD). *In vitro* and *in vivo* studies show that PGC-1α deficiency increases the susceptibility to MPTP-induced neurotoxicity, and enhanced PGC-1α levels protect neural cells from oxidative stress or α-synuclein-mediated neuronal death. Collective evidence to date, therefore, strongly suggests that PGC-1α is a promising therapeutic target within the central nervous system (CNS) for the treatment of PD. Herein we hypothesize that pharmacologically titrated *upregulation of PGC-1α will provide protection against neurodegeneration in PD*. Our goal is to modify disease progression by modulating PGC-1α levels with CNS-targeted small molecule therapeutics at an early stage of PD pathogenesis. We have identified that fenofibrate, an FDA approved drug, induces PGC-1α expression in CNS cells and exerts both cytoprotective and anti-inflammatory effects. Given the demonstrated safety profile of fenofibrate *in vivo*, we will test whether fenofibrate offers therapeutic potential in PD models as a prelude to a potential clinical study in patients.

Body

In **YEAR 1,** we have partially accomplished TO1. Elucidation of the mechanism through which fenofibrate regulates PGC-1α in primary CNS cells. We hypothesize that *fenofibrate enhances PGC-1α expression through enhanced transcription of the PGC-1α gene.* First, we have confirmed that the fenofibrate effect is PGC-1α-dependent using PGC-1α RNAi technique as well as PGC-1α knockout primary CNS cultures. Figure 1 showed that PGC-1α RNAi could inhibit approximately 36% of PGC-1α gene expression in BV2 cells (**Figure 1A**). The partial knockdown of PGC-1α gene expression offset fenofibrate-mediated anti-inflammation effect by 71% (**Figure 1B**).
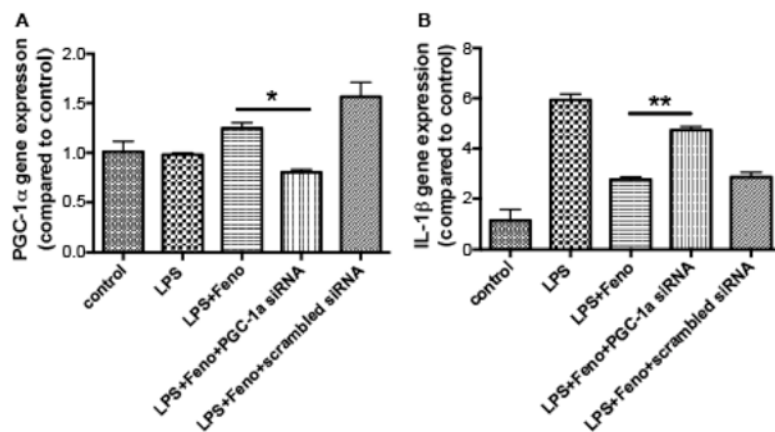

Figure 1. PGC-1α mediates the fenofibrate anti-inflammatory effect in BV2 cells. BV2 cells were incubated with 20nM siRNA targeting PGC-1a for 4 hrs followed by 20 uM fenofibrate for another 18 hrs. Then the cells were treated with 100 ng/mL LPS for 1 hr. Total RNA were extracted for RT-PCR to determined PGC-1α (A) and IL-1β (B) mRNA expression. *p<0.05, **p<0.01, One way ANOVA followed by Bonferroni multiple comparisons test.

We further confirm the important role of PGC-1α in regulating fenofibrate-mediated anti-inflammation effect using PGC-1α heterozygous knockout primary astrocytes. Figure 2 showed that high dose of fenofibrate (20 uM) was able to inhibit LPS-induced IL-1β expression by 68% in wild type primary astrocytes (**Figure 2A**) while by 51% in heterozygous astrocytes (**Figure 2B**), suggesting that PGC-1α knockout may attenuate the anti-inflammation effects of fenofibrate. We will further validate these findings using PGC-1α homozygous knockout primary cultures, which may provide more definite evidence.
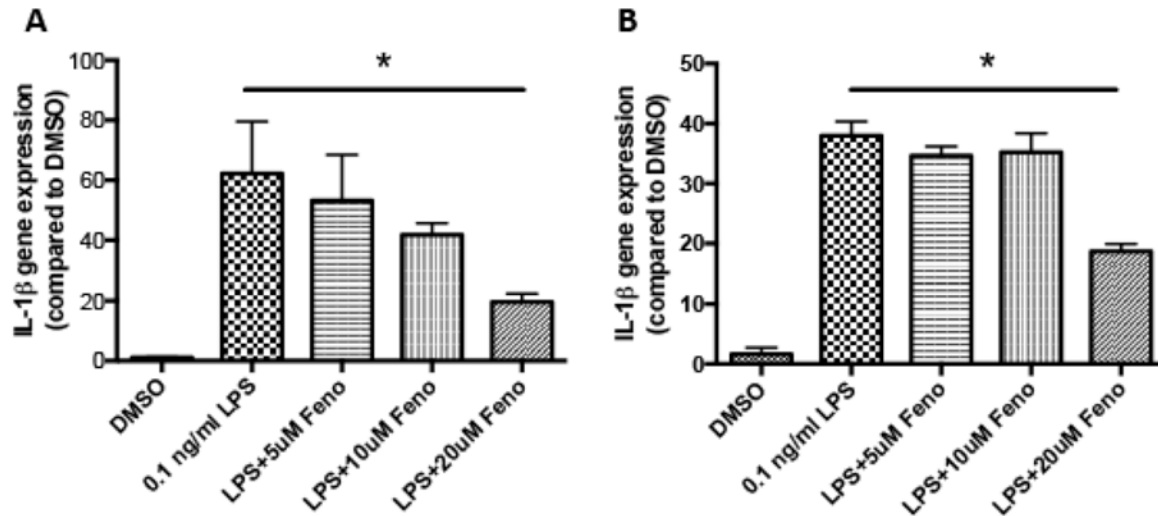

Figure 2. PGC-1α mediates the fenofibrate anti-inflammatory effect in primary astrocytes. Wild type (A) and PGC-1α heterozygous knockout (B) primary astrocytes were incubated with 5, 10 or 20 uM fenofibrate for 18 hrs. Then the cells were treated with 100 ng/mL LPS for 1 hr. Total RNA were extracted for RT-PCR to determined IL-1β mRNA expression. $*p<0.05$, One way ANOVA followed by Bonferroni multiple comparisons test.

Second, we evaluated whether fenofibrate-mediated anti-inflammatory effects are independent of PPARα, a known mediator of fenofibrate action in reducing cholesterol and triglycerides in the blood. We used PPARα RNAi technique to confirm that fenofibrate-mediated anti-inflammation effects are PPARα independent. Figure 3A and 3B showed that PPARα specific siRNA could inhibit PPARα gene and protein expression in a dose-dependent manner in primary astrocytes. As 10 nM PPARα siRNA could inhibit PPARα gene expression by more than 80% without obvious cytotoxicity, we chose 10 nM PPARα siRNA for the subsequent study to evaluate the role of PPARα in mediating the anti-inflammation effect of fenofibrate. Our data showed that while 10 nM PPARα siRNA could consistently inhibit 80% of PPARα gene expression in astrocytes under different treatment conditions compared to scramble siRNA (**Figure 3C**), fenofibrate can still significantly attenuate LPS-induced IL-1β expression in astrocytes with 80% of PPARα knockdown to a similar degree as without PPARα knockdown (**Figure 3D**). These results strongly suggest that fenofibrate mediate anti-inflammation in astrocytes in a PPARα-independent manner.
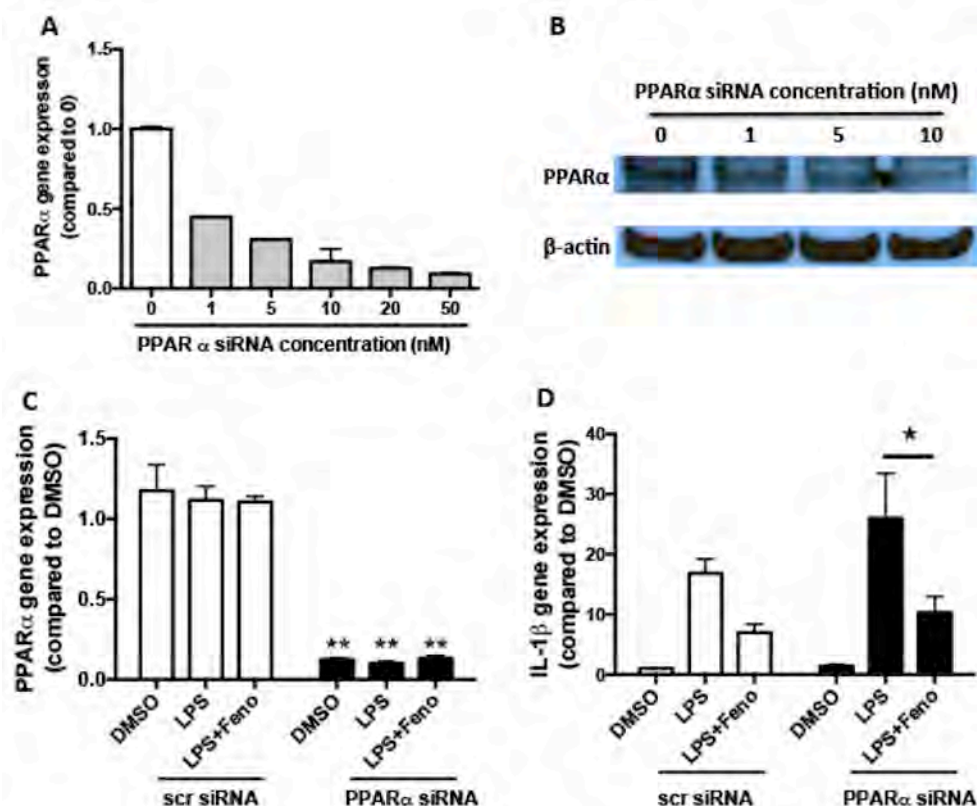
Figure 3. Fenofibrate mediate PPARα-independent anti-inflammation effect in primary astrocytes. Different concentrations of PPARα siRNA were added to primary astrocytes for 48 hrs. Total RNA and protein was collected. PPARα gene expression was determined by qRT-PCR (A) and protein expression was determined by western blot analysis (B). Then we used 10 nM PPARα siRNA for the subsequent experiments. Primary astrocytes were treated with 10 nM PPARα siRNA or scramble siRNA for 30 hrs followed by 20 uM fenofibrate for another 18 hrs. Then the cells were induced with 0.1 ng/ml LPS for 1 hr. Total RNA was extracted for PPARα (C) and IL-1β (D) gene expression.

To define the signaling pathways involved in fenofibrate-mediated anti-inflammation in CNS cells, we next performed microarray study on BV2 and primary astrocytes to identify relevant signaling molecules that regulate fenofibrate effects. BV2 cells or primary astrocytes were treated with fenofibrate or DMSO for 18 hrs followed by 1-hr LPS induction. Total RNA was collected for microarray study. The results will be reported in the next reporting cycle.

In **YEAR 1, we have partially accomplished** TO2. Evaluation of the neuroprotective effects of fenofibrate as mediated by PGC-1α up-regulation in a MPTP toxicant mouse model of PD. To test the hypothesis that *PGC-1α augmentation, or correction of PGC-1α deficiency, attenuates neurotoxicant-induced mitochondrial dysfunction, oxidative stress, and neuroinflammation*, we evaluated the *in vivo* preventive and protective efficacy of fenofibrate in a subchronic MPTP mouse model of early PD.

For *protective intervention*, animals will first be subjected to MPTP intoxication and then receive drug treatment. 2-month old C57 mice received 5-day MPTP i.p. injection (30mg/kg) or saline followed by 14-day fenofibrate treatment (i.p., 100mg/kg). Then these animals were sacrificed for tyrosine hydroxylase (TH) immunohistochemistry analysis. 5-day MPTP subchronic treatment induced significant TH neuronal cell loss in the nigra region, while subsequent fenofibrate treatment could slightly protect TH-positive cell loss from MPTP neurotoxicity (**Figure 4**). Similarly, MPTP induced significant loss of TH positive fibers in the striatum and fenofibrate could slightly attenuate the loss in these animals but didn't reach significance (**Figure 5**). The results of the MPTP *in vivo* study indicated that fenofibrate had the potential to protect neurons against MPTP-induced oxidative stress.
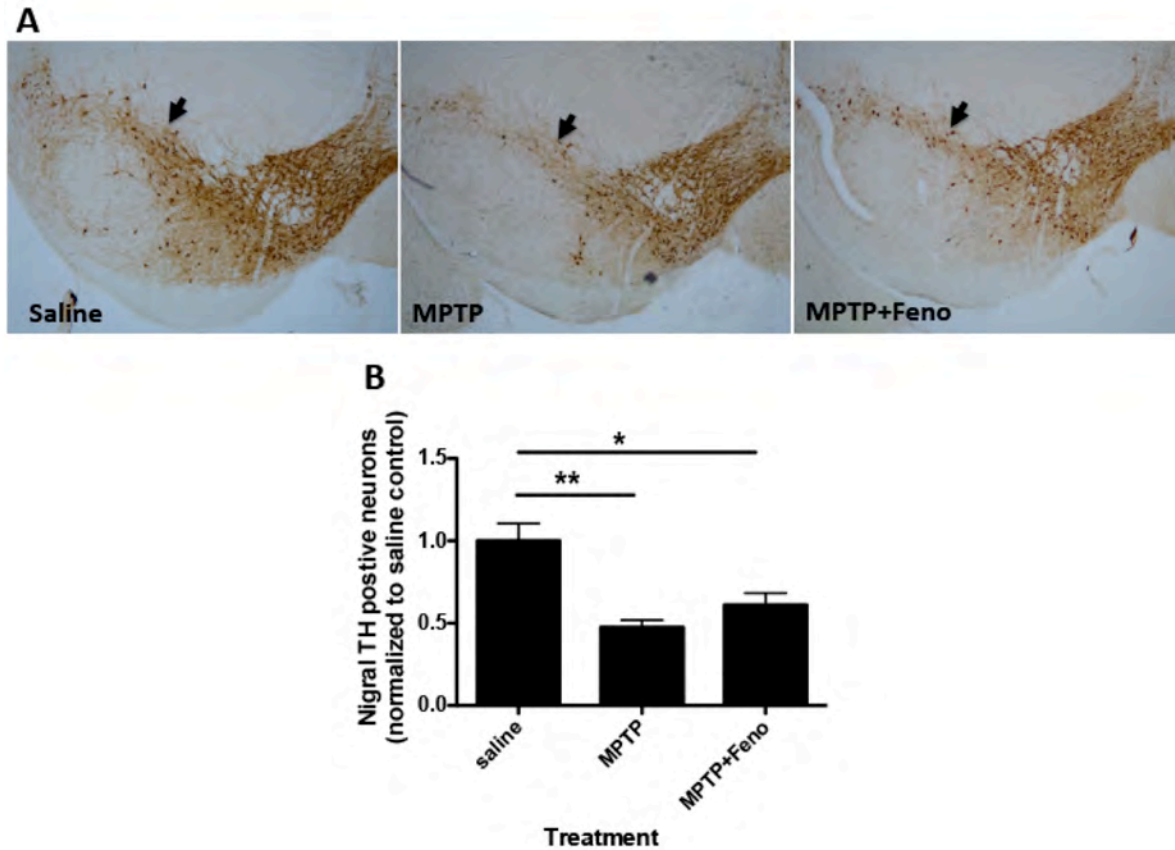

Figure 4. Fenofibrate treatment in MPTP-intoxicated mice. 2-month old C57 mice received 5-day MPTP i.p. injection (30mg/kg) or saline followed by 14-day fenofibrate treatment (i.p., 100mg/kg). Then these animals were sacrificed for tyrosine hydroxylase (TH) immunohistochemistry analysis. Panel A are the representative TH stained images of the nigral sections in the saline control, MPTP and MPTP plus fenofibrate treatment groups. Panel B is the quantification of TH positive neurons in the substantia nigra.*, P,0.05, **, p<0.01, Student t test.
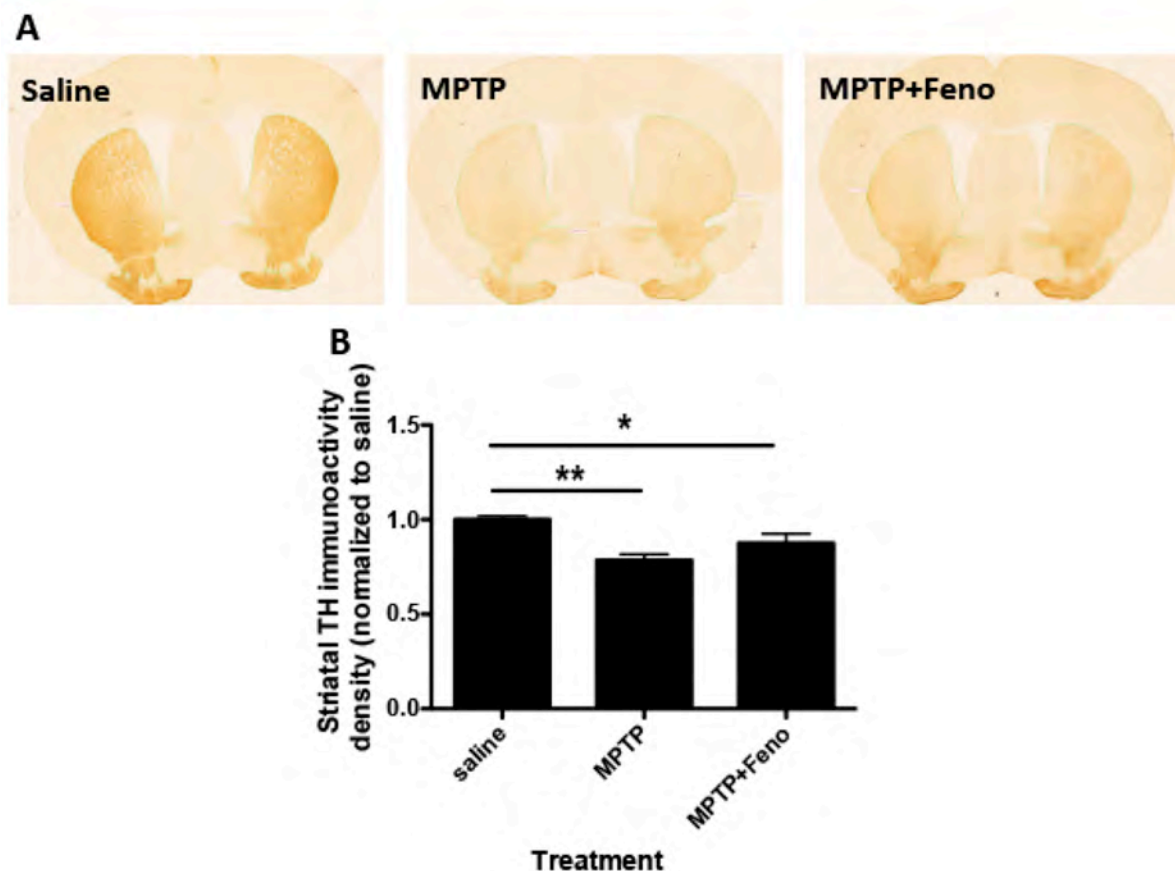
Figure 5. Fenofibrate treatment in MPTP-intoxicated mice. 2-month old C57 mice received 5-day MPTP i.p. injection (30mg/kg) or saline followed by 14-day fenofibrate treatment (i.p., 100mg/kg). Then these animals were sacrificed for tyrosine hydroxylase (TH) immunohistochemistry analysis. Panel A are the representative TH stained images of the striatal sections in the saline control, MPTP and MPTP plus fenofibrate treatment groups. Panel B is the quantification of optical density of TH positive fibers in the striatum. *, P,0.05, **, p<0.01, Student t test.

Next, we need to optimize the fenofibrate treatment paradigms to enhance the therapeutic efficacy of fenofibrate. As fenofibrate is generally taken with meals in patients to help better absorb the medicine, we made special chow food containing 0.2% w/w fenofibrate. We treat a group of 2-month C57 mice (n=8) with fenofibrate chow or control regular chow for 4 weeks. Then these animals were sacrificed, blood, liver and brain (nigra) tissues were collected for qRT-PCR analysis. We found that the expression of PGC-1α gene was significantly increased in the liver and nigra (**Figure 5B-5C**). We further measured the gene expression of tyrosine hydroxylase (TH), nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the nigral tissues to determine the possible therapeutic effects of fenofibrate on the brains. Interestingly, the gene expression of NGF was significantly increased in the group with fenofibrate treatment while the expression of TH and BDNF was slightly increased in these animals (**Figure 5D-5F**). These finding suggest that fenofibrate might activate a possible NGF/PGC-1α signaling pathway in CNS. Previous studies showed that astrocytic NGF can activate mitochondrial PGC-1α expression in neurons [1]. In the future, we will further investigate this potential signaling pathway using PGC-1α knockout mice.
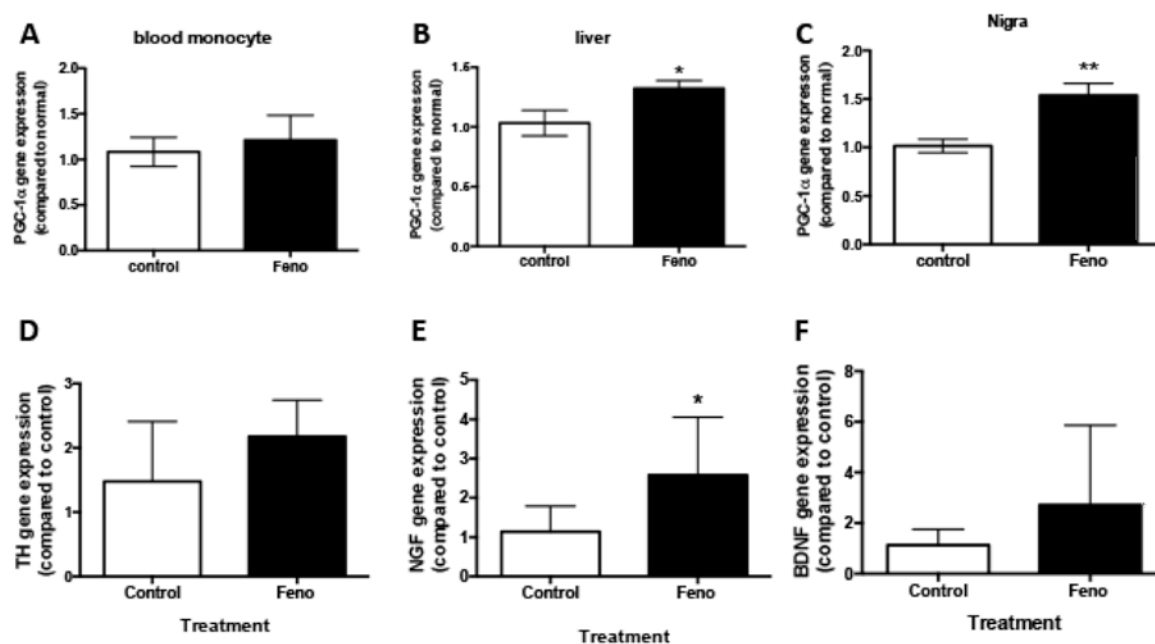
Figure 5. Gene expression profiles in mice with fenofibrate treatment. 2–month old C57 mice received either special chow with 0.2% w/w fenofibrate or regular control chow for 4 weeks (n=8). Then these animals were sacrificed and blood, liver and nigral tissues were extracted for qRT-PCR. PGC-1a gene expression in the blood monocyte (A), liver (B), and nigral tissue (C) were determined by qRT-PCR. TH (D), NGF (E), and BDNF (F) gene expression in the nigral tissue was determined by qRT-PCR. *, $p < 0.05$, Student's t test.

Key Research Accomplishments:
1. Determine that fenofibrate-mediated anti-inflammation effects in CNS cells is PGC-1α dependent
2. Determine that fenofibrate-mediated anti-inflammation effects in CNS cells is PPARα independent
3. Demonstrate a potential neuroprotection effect of fenofibrate in a MPTP mouse model
4. Demonstrate a potential NGF/PGC-1α signaling pathway in response to fenofibrate *in vivo*

Reportable Outcomes
1. Applied for an international patent application for using fenofibrate as the treatment for Parkinson's disease and related neurodegenerative disease.
2. Manuscript under preparation: Wang J, Su X, and Federoff HJ. "Fenofibrate-mediated PGC-1a activation is a therapeutic strategy for the treatment of Parkinson's disease"

Conclusion
In conclusion, we have made substantial progress on this project. We met the technical objectives that were originally outlined for **Year 1**. Importantly we have established the *in vitro* (primary cultures) and *in vivo* models (MPTP mouse model) needed to complete this

project. We identified some potential signaling pathways that may be involved in the fenofibrate-mediated anti-inflammation and neuroprotection. In the next year, we will use microarray technique to systematically investigate the signaling pathways in CNS cells in response to fenofibrate treatment. We will also evaluate some new fenofibrate treatment paradigms in our MPTP mouse model.

Reference:

1. Chen LW, Horng LY, Wu CL, Sung HC, Wu RT (2012) Activating mitochondrial regulator PGC-1alpha expression by astrocytic NGF is a therapeutic strategy for Huntington's disease. Neuropharmacology 63: 719-732.